c) a nucleic acid/nucleic acid denaturing reagent permitting the formation of a PNA probe/nucleic acid complex when said selected target sequence is present;

d) a detection zone; and

e) [a] said separation zone in communication with [to] said introduction zone and said detection zone.

## REMARKS

Claims 32-67 are pending in this application. Claims 32-67 are amended herein. No new matter has been added. Claims 32-67 are presented for reconsideration.

Amendments to the specification are made to comply with rule § 1.77(4), correct typographical errors, or restore disclosure from deleted claims (i.e., claim 9 for insert to page 11 of the specification above). No new matter has been added.

***Claims Rejections Under 35 U.S.C. §112, First Paragraph***

Claim 38, 54 and 66 are rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter in the recitation of the limitation "particle." The Office Action admits that the specification mentions two embodiments of particles, colored and resin. Nonetheless, it asserts that no written description of the generic limitation "particle" is given. Applicants respectfully traverse this rejection.

Applicants have described at page 5, line 7 that the PNA probes may be labeled with colored particles. Further, Applicants described resin-bound PNA probes, as noted in the Office Action. This provides the sufficient support for the term "particles" as both these embodiments have the same inherent physical property of being particles. See MPEP §2163.07(a). Applicants have provided for a broad disclosure of detectable moieties. At page 9, line 11, Applicants have indicated that the detectable moieties encompassed by the present invention include but are not limited to the recited embodiments. Thus this invention clearly encompasses within its scope the subgenus particle as a detectable moiety. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 39, 55, and 64 are rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter in the recitation of the limitation "charge-modifying moiety." Applicants respectfully traverse this basis of rejection.

Applicants have described how to use charge-modifying moieties in the present invention at page 15, lines 21-30. In that passage of the specification, Applicants first propose that charge-modifying moieties may be used to increase the resolution of the separation of the DNA/PNA complexes by increasing the force experienced by these complexes in an electric field. The specification recites that "the second binding partner may comprise a charge-modifying moiety" (the term "binding partner" referring to a PNA probe). Thus the claims 39, 55, and 64 are fully supported by the specification and do not constitute new matter. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 46-67 are rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter in the recitation of the limitation of a denaturing reagent in step c). Applicants respectfully traverse this basis of rejection.

Applicants describe the use of denaturing medium and reagents throughout the specification. For example, at page 10, lines 10-17, page 11. lines 9-13, page 13, lines 3-11, page 14, lines 6-12, page 15, line 9, page 25, lines 5 & 16, and page 28, lines 17-24. Of these, cited passages of pages 14, 15, 25, and 28 mention the use of denaturing medium such as elevated temperature or denaturing reagents used in conjunction with electrophoretic separation methods. Passages of pages 25 and 28, describe the use of denaturing conditions, such as elevated temperatures or 30% formamide buffer, in capillary electrophoresis. Thus step c) of claims 46-67 is fully supported by the specification as filed. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 58-67 are rejected under 35 U.S.C. §112, first paragraph as allegedly containing new matter in the recitation in step b) of both, a double stranded polynucleotide and at least one PNA probe as it requires the PNA probe to be present both in the sample and in the apparatus. Applicants have amended the claims to clarify the subject matter regarded as their invention. Claim 58 has been amended to recite "at least one PNA probe disposed to mix upstream of a separation zone with a sample introduced in each said introduction zone, said sample comprising at least one double stranded polynucleotide, said at least one PNA probe having a sequence complementary to a selected target

sequence suspected to be present in said at least one double stranded polynucleotide." Applicants submit that this amendment obviates this rejection.

Claims 36 and 46-67 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, the Office Action asserts that in part b) of claim 58, the wording is unclear as to whether the sample contains both a double stranded polynucleotide and a PNA probe which is separate from the PNA probe that is in the apparatus and is disposed to mix with the sample, or whether the sample only contains a double stranded nucleotide. Applicants have amended the claims to clarify and distinctly claim that the PNA probe is disposed to mix with the sample comprising at least one double stranded polynucleotide, and to characterize the probe as having a sequence complementary to a selected target sequence suspected to be present in the at least one double stranded polynucleotide. Applicants submit that the amended claim satisfies this requirement. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Office Action further asserts that the recitation of "the medium" in claim 36 does not have antecedent basis. This claim has been amended to recite: "adjusting the temperature of the mixture resulting from step b." Applicants submit that the amended claim satisfies this requirement. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Office Action also asserts that the metes and bounds of claim 46-67 are vague and indefinite in that there are two possible interpretations of the wording of subsection b), i.e., the mixing of the PNA probe with the sample is either performed in the introduction zone or in any zone or channel of the apparatus. Applicant respectfully traverse this basis of rejection.

The wording of claim 46 regarding the PNA probe disposed to mix with the sample is not vague and indefinite but broad so as to encompass all the locations that would meet the limitations of the claim, particularly element d). For the proper operation of the claimed apparatus, it is not critical where the PNA probe is located for mixing with the sample, so long as the mixing occurs upstream of the separation. Accordingly, element d) recites the limitation "said separation zone separating said

PNA/nucleic acid complexes from other components present in said introduction zone and said separation zone."

Nonetheless, to speed prosecution of this application, Applicants have amended the claim element b) to specify that the PNA probe is disposed to mix upstream of a separation zone with a sample. This language is intended to cover all embodiments that provide for the mixing of the PNA probe with the sample at a time prior to the mixture entering the separation zone. Applicants submit that the remarks and the amendment to the claim overcome this basis of rejection. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Office Action asserts that the recitation in claim 46 of "An apparatus for detecting..." without reciting a detection zone renders the claim vague and indefinite. Applicants have amended the claim by deleting in the preamble the language "for detecting at least one selected target sequence in at least one polynucleotide, said apparatus." This amendment overcomes this basis of rejection. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Office Action points to the language "at least PNA probe" in claim 58 as being unclear. Applicants have amended this language to recite "at least one PNA probe." This amendment overcomes this basis of rejection. Accordingly, Applicants respectfully request that this rejection be withdrawn.

***Claim Rejections Under 35 U.S.C. §102(b)***

Claims 32, 33, 35-37, 39, 40, 42-47, 50-53, 55 and 56 are rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Rose. The Office Action asserts that Rose discloses the separation of PNA/nucleic acid complexes from hybridized nucleic acid/nucleic acid duplexes by capillary electrophoresis as well as capillary electrophoretic apparatus for performing the separation such as claimed. The Office Action remarks that, in Figure 7, on page 3549, Roses discloses PNA that displaces complementary oligonucleotide from a DNA duplex and is being separated from the other components in the mixture. It further alleges that the buffer conditions inherently contain denaturing reagents. Applicants respectfully traverse this basis of rejection.

Contrary to the Office Action's assertion, Rose does not disclose the use of denaturing reagents when both a DNA duplex and a labeled PNA probe are present in a mixture containing a sample

polynucleotide with its complementary strand prior to separating the resulting components of the mixture as claimed in the instant application. In Figure 7, Rose exemplifies the strand displacement ability of short PNA probes. It is apparent from Figure 7 that no denaturing reagents or conditions were used in that experiment since the oligonucleotide duplex (ODN/ODN) is mostly observed. To the contrary, Figure 7 shows that, under such conditions, very few of the heteroduplex (PNA/ODN) is formed even after two hours or more. Only an amount of single stranded oligonucleotide (ODN) equaling the amount of heteroduplex (PNA/ODN) is observed evidencing no spontaneous dissociation of the oligonucleotide duplex. The formation of single stranded oligonucleotide is thus only due to PNA strand displacement, not to denaturing conditions or reagents.

Unlike in the present application, Rose discloses the formation of the PNA/ODN complex which occurs from the ability of PNA to displace one ODN strand from the ODN duplex; this process is highly disfavored, even on short double stranded nucleic acid. As acknowledged by Rose, the equilibrium PNA + ODN/ODN $\leftrightarrow$ ODN + PNA/ODN is strongly in favor of the homoduplex, and the rate of formation of the heteroduplex is much slower when double-stranded ODN is used as starting material than when only single stranded ODN is used (page 3549, col. 2, lines 17-22). Thus, in making these observations, Rose rather teaches away from making PNA/DNA heteroduplexes starting from double-stranded DNA.

In addition, contrary to the Office Action's assertion, Rose does not teach denaturing conditions for denaturing nucleic acid/nucleic acid duplexes while permitting formation of PNA/nucleic acid heteroduplexes. Rose mentions the use of elevated temperature in one experiment, page 3547, Figure 3. However, no complementary strand of ODN is present in that experiment. Further, the heteroduplex PNA/ODN is dissociated upon heat application. Therefore, Applicants believe that the denaturing conditions disclosed by Rose do not meet the instantly claimed limitations that require the denaturation of double-stranded polynucleotide sample while permitting the formation of PNA/nucleic acid complexes.

Therefore, the Rose reference does neither anticipate or suggest the claimed invention. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 32-39, 44, and 45 are rejected under 35 USC §102 (b) and (e) as being allegedly anticipated by US patent 5,217,866 to Summerton *et al.* (Summerton). The Office Action asserts that

Summerton discloses hybridization assay procedures wherein PNA polymers replace nucleic acid probes targeted to single stranded sample nucleic acid. Applicants respectfully traverse this basis of rejection.

The methods of the instant invention concern the use of labeled PNA probes for use in diagnostic assays. These labeled PNA probes offer certain advantages over the prior art. One advantage is that they bind strongly to polynucleotides so that they can anneal to a complementary polynucleotide sequence in the presence of the complementary polynucleotide strand and under conditions that are denaturing to double stranded polynucleotides. Another advantage is that the PNA probes are labeled with detectable moieties so as to become detectable.

Unlike the instant methods, the methods disclosed in Summerton use labeling reagents separate from the PNA probes, such as dansylspermine, for detection of the bound analyte (Figure 14 and col. 23, lines 5-67). Further, these labeling reagents are designed to bind to the polynucleotide's phosphate groups by electrostatic interactions. Thus, these methods differ from the presently claimed methods in that the detectable moieties are not bound to the PNA probes. Also, in Summerton, the use of labeling reagents separate from the probes requires a supplemental separation step to remove the unbound labeling reagents from the labeled complex. In the instant methods, such step is avoided as the separation of the probe bound to the polynucleotide from the unbound probe occurs simultaneously from the separation of the labeled complex from the unlabeled complex. This is achieved by the use of labeled PNA probes.

Therefore, the methods of the present invention are neither anticipated or suggested by Summerton. Accordingly, Applicants respectfully request that this rejection be withdrawn.

***Claim Rejections under 35 USC §103(a).***

Claims 46, 49-58 and 61-67 are rejected under 35 USC §103(a) as being unpatentable over US patent 5,498,392 to Wilding *et al.* (Wilding) taken in view of US patent 5,217,866 to Summerton *et al.* (Summerton). Specifically, the Office Action asserts that Wilding discloses an apparatus which is a microchip device for nucleic acid manipulations including polymerase chain reaction (PCR) and detection of PCR products. The Office Action further asserts that the detection of PCR products is motivated and suggested in Wilding as being via hybridization techniques known in the art. The Office

Action also asserts that the purification of a polynucleotide bead-conjugated probe that is bound to an target polynucleotide in a separation zone is suggested and motivated in Wilding since purification includes the separation of components from each other. The Office Action also asserts that it would have been obvious to use the hybridization assay with PNA polymers disclosed in Summerton in the apparatus of Wilding given the motivation to use known hybridization assays in Wilding and the prior disclosure of PNA hybridization assays by Summerton. Applicants respectfully traverse this basis of rejection.

Wilding is directed to microscale apparatus for handling nucleic acid reactions such as PCR and/or purification of products from these reactions. However, Wilding only teaches or suggests using these apparatus for assays or reactions known in the prior art. Specifically, Wilding does not disclose apparatus for assays comprising labeled PNA probes that are bound to polynucleotides such as claimed in the instant application.

Summerton does not cure the defects of Wilding. As shown above, Summerton does not teach or suggest assays using labeled PNA probes that are bound to polynucleotides. Summerton only teach assays using separate labeling reagents. Thus, Wilding, even in view of Summerton, does not provide the necessary motivation to modify the apparatus disclosed therein to arrive at the claimed invention. Thus, Applicants submit that claims 46, 49-58 and 61-67 are patentable over Wilding in view of Summerton. Accordingly, Applicants respectfully request that this rejection be withdrawn.

Claims 32-37, 39-53, 55 and 56 are rejected under 35 USC §103(a) as being unpatentable over Rose taken in view of Chen *et al.* (Ref. CN). Applicants respectfully traverse this basis of rejection.

Applicants have shown above that Rose does not teach or suggest assay conditions that are denaturing to nucleic acid duplexes while permitting the formation of PNA/nucleic acid heteroduplexes such as instantly claimed.

Chen does not cure the defects of Rose, because it does not teach or suggest assay conditions that are denaturing to nucleic acid duplexes while permitting the formation of PNA/nucleic acid heteroduplexes such as instantly claimed. Chen merely teach hybridization procedures for nucleic acids. Chen does not teach or suggest that these hybridization procedures would be suitable for the formation of PNA/nucleic acid heteroduplexes, less in the presence of the complementary nucleic acid strand.

Thus, Chen does not provide the necessary motivation to modify the Rose teachings such that denaturing conditions for nucleic acids duplexes permitting the formation of PNA/nucleic acid duplexes would be obtained. Therefore, the instant claims are patentable over Rose in view of Chen. Accordingly, Applicants respectfully request that this rejection be withdrawn.

## CONCLUSION

Applicants believe that, in view of the instant amendments to the claims and the remarks made herein, the claims are in condition for allowance. Accordingly, Applicants respectfully request prompt favorable action on reconsideration of this application.

The Examiner is cordially invited to contact Applicants' undersigned representative at (617) 248-7738 for a rapid prosecution of this application.

Respectfully submitted,

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